<110> Wood, Keith

Hannah, Rita

Moravec, Richard A

<120> IMPROVED METHOD FOR DETECTION OF ATP

<130> 10743/6

<140> US 09/813,279

<141> 2001-03-19

<150> US60/269,526

<151> 2001-02-16

<160> 8

<170> PatentIn version 3.1

<210> 1

<211> 544

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant of LucPpe2 luciferase

<400> 1

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Ala	Asp	Gly	Thr 20	Ala	Gly	Glu	Gln	Met 25	Phe	Asp	Ala	Leu	Ser 30	Arg	Ту
Ala	Asp	Ile 35	Ser	Gly	Cys	Ile	Ala 40	Leu	Thr	Asn	Ala	His 45	Thr	Lys	Gl
Asn	Val 50	Leu	Tyr	Glu	Glu	Phe 55	Leu	Lys	Leu	Ser	Cys 60	Arg	Leu	Ala	Gl

Ser Glu Asn Gly Leu Gln Phe Phe Leu Pro Val Ile Ala Ser Leu Tyr

Ser Phe Lys Lys Tyr Gly Leu Lys Gln Asn Asp Thr Ile Ala Val Cys

70 75

85

Leu Gly Ile Ile Ala Ala Pro Val Ser Asp Lys Tyr Ile Glu Arg Glu 100 105 110

Leu Ile His Ser Leu Gly Ile Val Lys Pro Arg Ile Ile Phe Cys Ser

Lys Asn Thr Phe Gln Lys Val Leu Asn Val Lys Ser Lys Leu Lys Ser 130 135 140

Val Glu Thr Ile Ile Ile Leu Asp Leu Asn Glu Asp Leu Gly Gly Tyr 145 150 155 160

Gln Cys Leu Asn Asn Phe Ile Ser Gln Asn Ser Asp Ser Asn Leu Asp 165 170 175

Val Lys Lys Phe Lys Pro Tyr Ser Phe Asn Arg Asp Asp Gln Val Ala 180 185 190

Leu Val Met Phe Ser Ser Gly Thr Thr Gly Val Pro Lys Gly Val Met 195 200 205

Leu Thr His Lys Asn Ile Val Ala Arg Phe Ser Leu Ala Lys Asp Pro 210 215 220

Thr Phe Gly Asn Ala Ile Asn Pro Thr Thr Ala Ile Leu Thr Val Ile 225 230 235 240

Pro Phe His His Gly Phe Gly Met Met Thr Thr Leu Gly Tyr Phe Thr 245 250 255

Cys Gly Phe Arg Val Val Leu Met His Thr Phe Glu Glu Lys Leu Phe 260 265 270

Leu Gln Ser Leu Gln Asp Tyr Lys Val Glu Ser Thr Leu Leu Val Pro 275 280 285

Thr Leu Met Ala Phe Leu Ala Lys Ser Ala Leu Val Glu Lys Tyr Asp 290 295 300

Leu Ser His Leu Lys Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser Lys 305 310 315 320

Glu Ile Gly Glu Met Val Lys Lys Arg Phe Lys Leu Asn Phe Val Arg 325 330 335

Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Val Leu Ile Thr Pro 340 345 350

Ala Val Lys Val Val Asp Pro Thr Thr Gly Lys Ile Leu Gly Pro Asn 370 375375380

Glu Pro Gly Glu Leu Tyr Phe Lys Gly Ala Met Ile Met Lys Gly Tyr 385 390 395 400

Tyr Asn Asn Glu Glu Ala Thr Lys Ala Ile Ile Asp Asn Asp Gly Trp \$405\$

Leu Arg Ser Gly Asp Ile Ala Tyr Tyr Asp Asn Asp Gly His Phe Tyr \$420\$ \$425\$ \$430

Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln Val

Ala Pro Ala Glu Ile Glu Gly Ile Leu Leu Gln His Pro Tyr Ile Val 450 455 460 Ser Glu Asn Gly Leu Gln Phe Phe Leu Pro Val Ile Ala Ser Leu Tyr 85 90 95

Leu Gly Ile Ile Val Ala Pro Val As
n Asp Lys Tyr Ile Glu Arg Glu 100 \$105\$ 110

Leu Ile His Ser Leu Gly Ile Val Lys Pro Arg Ile Val Phe Cys Ser 115 120 125

Lys Asn Thr Phe Gln Lys Val Leu Asn Val Lys Ser Lys Leu Lys Ser 130 \$135\$

Ile Glu Thr Ile Ile Ile Leu Asp Leu Asn Glu Asp Leu Gly Gly Tyr 145 150 155 160

Gln Cys Leu Asn Asn Phe Ile Ser Gln Asn Ser Asp Ser Asn Leu Asp 165 170 175

Val Lys Lys Phe Lys Pro Tyr Ser Phe Asn Arg Asp Asp Gln Val Ala 180 185 190

Leu Ile Met Phe Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met 195 200205

Leu Thr His Lys Asn Ile Val Ala Arg Phe Ser Leu Ala Lys Asp Pro 210 215 220

Thr Phe Gly Asn Ala Ile Asn Pro Thr Thr Ala Ile Leu Thr Val Ile 225 230 235 240

Pro Phe His His Gly Phe Gly Met Met Thr Thr Leu Gly Tyr Phe Thr 245 250 255

Cys Gly Phe Arg Val Val Leu Met His Thr Phe Glu Glu Lys Leu Phe 260 265 270

Leu Gln Ser Leu Gln Asp Tyr Lys Val Glu Ser Thr Leu Leu Val Pro 275 280 285

Thr Leu Met Ala Phe Leu Ala Lys Ser Ala Leu Val Glu Lys Tyr Asp 290 295 300

Leu Ser His Leu Lys Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser Lys

305					310					315					320
Glu	Ile	Gly	Glu	Met 325	Val	Lys	Lys	Arg	Phe 330	Lys	Leu	Asn	Phe	Val 335	Arg
Gln	Gly	Tyr	Gly 340	Leu	Thr	Glu	Thr	Thr 345	Ser	Ala	Val	Leu	Ile 350	Thr	Pro
Lys	Gly	Asp 355	Ala	Lys	Pro	Gly	Ser 360	Thr	Gly	Lys	Ile	Val 365	Pro	Phe	His
Ala	Val 370	Lys	Val	Val	Asp	Pro 375	Thr	Thr	Gly	Lys	Ile 380	Leu	Gly	Pro	Asn
Glu 385	Pro	Gly	Glu	Leu	Tyr 390	Phe	Lys	Gly	Pro	Met 395	Ile	Met	Lys	Gly	Tyr 400
Tyr	Asn	Asn	Glu	Glu 405	Ala	Thr	Lys	Ala	Ile 410	Ile	Asp	Asn	Asp	Gly 415	Trp
Leu	Arg	Ser	Gly 420	Asp	Ile	Ala	Tyr	Tyr 425	Asp	Asn	Asp	Gly	His 430	Phe	Tyr
Ile	Val	Asp 435	Arg	Leu	Lys	Ser	Leu 440	Ile	Lys	Tyr	Lys	Gly 445	Tyr	Gln	Val
Ala	Pro 450	Ala	Glu	Ile	Glu	Gly 455	Ile	Leu	Leu	Gln	His 460	Pro	Tyr	Ile	Val
Asp 465	Ala	Gly	Val	Thr	Gly 470	Ile	Pro	Asp	Glu	Ala 475	Ala	Gly	Glu	Leu	Pro 480
Ala	Ala	Gly	Val	Val 485	Val	Gln	Thr	Gly	Lys 490	Tyr	Leu	Asn	Glu	Gln 495	Ile
Val	Gln	Asp	Tyr 500	Val	Ala	Ser	Gln	Val 505	Ser	Thr	Ala	Lys	Trp 510	Leu	Arg
Gly	Gly	Val 515	Lys	Phe	Leu	Asp	Glu 520	Ile	Pro	Lys	Gly	Ser 525	Thr	Gly	Lys
Ile	Asp 530	Arg	Lys	Val	Leu	Arg 535	Gln	Met	Phe	Glu	Lys 540	His	Thr	Asn	Gly

<210> 3

<211> 544

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant of LucPpe2 luciferase

<400> 3

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Met Ala Asp Lys Asn Ile Leu Tyr Gly Pro Glu Pro Phe Tyr Pro Leu 1 5 10 15

Glu Asp Gly Thr Ala Gly Glu Gln Met Phe Asp Ala Leu Ser Arg Tyr 20 25 30

Ala Asp Ile Pro Gly Cys Ile Ala Leu Thr Asn Ala His Thr Lys Glu 35 40 45

Asn Val Leu Tyr Glu Glu Phe Leu Lys Leu Ser Cys Arg Leu Ala Glu 50 55 60

Ser Phe Lys Lys Tyr Gly Leu Lys Gln Asn Asp Thr Ile Ala Val Cys 70 75 80

Ser Glu Asn Ser Leu Gln Phe Phe Leu Pro Val Ile Ala Ser Leu Tyr 85 90 95

Leu Gly Ile Ile Val Ala Pro Val Asn Asp Lys Tyr Ile Glu Arg Glu 100 105 110

Leu Ile His Ser Leu Gly Ile Val Lys Pro Arg Ile Val Phe Cys Ser 115 120 125

Lys Asn Thr Phe Gln Lys Val Leu Asn Val Lys Ser Lys Leu Lys Ser 130 135 140

- Gln Cys Leu Asn Asn Phe Ile Ser Gln Asn Ser Asp Ser Asn Leu Asp 165 170 175
- Val Lys Lys Phe Lys Pro Tyr Ser Phe Asn Arg Asp Asp Gln Val Ala 180 185 190
- Leu Ile Met Phe Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met 195 200205
- Leu Thr His Lys Asn Ile Val Ala Arg Phe Ser Ile Ala Lys Asp Pro 210 215 220
- Thr Phe Gly Asn Ala Ile Asn Pro Thr Ser Ala Ile Leu Thr Val Ile 225 230 235 240
- Pro Phe His His Gly Phe Gly Met Met Thr Thr Leu Gly Tyr Phe Thr 245 250 255
- Cys Gly Phe Arg Val Val Leu Met His Thr Phe Glu Glu Lys Leu Phe 260 265 270
- Leu Gln Ser Leu Gln Asp Tyr Lys Val Glu Ser Thr Leu Leu Val Pro 275 280 285
- Thr Leu Met Ala Phe Leu Ala Lys Ser Ala Leu Val Glu Lys Tyr Asp 290 295 300
- Leu Ser His Leu Lys Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser Lys 305 310 315 320

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- Glu Ile Gly Glu Met Val Lys Lys Arg Phe Lys Leu Asn Phe Val Arg 325 330 335
- Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Val Leu Ile Thr Pro 340 345 350
- Lys Gly Asp Ala Lys Pro Gly Ser Thr Gly Lys Ile Val Pro Phe His 355 360 365
- Ala Val Lys Val Val Asp Pro Thr Thr Gly Lys Ile Leu Gly Pro Asn 370 375 380

Glu Pro Gly Glu Leu Tyr Phe Lys Gly Pro Met Ile Met Lys Gly Tyr 385 390 395 400

Tyr Asn Asn Glu Glu Ala Thr Lys Ala Ile Ile Asp Asn Asp Gly Trp \$405\$ \$410\$ \$415\$

Leu Arg Ser Gly Asp Ile Ala Tyr Tyr Asp Asn Asp Gly His Phe Tyr 420 425 430

Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln Val 435 440 445

Ala Pro Ala Glu Ile Glu Gly Ile Leu Leu Gln His Pro Tyr Ile Val 450 455 460

Asp Ala Gly Val Thr Gly Ile Pro Asp Glu Ala Ala Gly Glu Leu Pro 465 470 475 480

Ala Ala Gly Val Val Val Gln Thr Gly Lys Tyr Leu Asn Glu Gln Ile 485 490

Val Gln Asp Tyr Val Ala Ser Gln Val Ser Thr Ala Lys Trp Leu Arg 500 505 510

Gly Gly Val Ile Phe Leu Asp Glu Ile Pro Lys Gly Ser Thr Gly Lys $515 \hspace{1.5cm} 520 \hspace{1.5cm} 525$

Ile Asp Arg Lys Val Leu Arg Gln Met Leu Glu Lys His Thr Asn Gly 530 540

<210> 4

<211> 544

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant of LucPpe2 luciferase

<400> 4

Met Ala Asp Lys Asn Ile Leu Tyr Gly Pro Glu Pro Phe Tyr Pro Leu

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Ala Ala Ile Pro Gly Cys Ile Ala Leu Thr Asn Ala His Thr Lys Glu $_{35}$ $_{40}$

Asn Val Leu Tyr Glu Glu Phe Leu Lys Leu Ser Cys Arg Leu Ala Glu 50 55 60

Ser Phe Lys Lys Tyr Gly Leu Lys Gln Asn Asp Thr Ile Ala Val Cys 65 70 75 80

Ser Glu Asn Ser Leu Gln Phe Phe Leu Pro Val Ile Ala Ser Leu Tyr 85 90 95

Leu Gly Ile Ile Val Ala Pro Val Asn Asp Lys Tyr Ile Glu Arg Glu 100 105 110

Leu Ile His Ser Leu Gly Ile Val Lys Pro Arg Ile Val Phe Cys Ser 115 120 125

Lys Asn Thr Phe Gln Lys Val Leu Asn Val Lys Ser Lys Leu Lys Ser 130 135 140

Gln Cys Leu Asn Asn Phe Ile Ser Gln Asn Ser Asp Ser Asn Leu Asp 165 170 175

Val Lys Lys Phe Lys Pro Tyr Ser Phe Asn Arg Asp Asp Gln Val Ala 180 185 190

Ser Ile Met Phe Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met 195 200 205

Leu Thr His Lys Asn Ile Val Ala Arg Phe Ser Ile Ala Lys Asp Pro 210 215 220

Thr Phe Gly Asn Ala Ile Asn Pro Thr Ser Ala Ile Leu Thr Val Ile 225 230 235 240 Pro Phe His His Gly Phe Gly Met Met Thr Thr Leu Gly Tyr Phe Thr 245 250 255

Cys Gly Phe Arg Val Val Leu Met His Thr Phe Glu Glu Lys Leu Phe 260 265 270

Leu Gln Ser Leu Gln Asp Tyr Lys Val Glu Ser Thr Leu Leu Val Pro 275 280 285

Thr Leu Met Ala Phe Leu Ala Lys Ser Ala Leu Val Glu Lys Tyr Asp 290 295 300

Leu Ser His Leu Lys Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser Lys 305 310 315 320

Glu Ile Gly Glu Met Val Lys Lys Arg Phe Lys Leu Asn Phe Val Arg 325 $$ 330 $$ 335

Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Val Leu Ile Thr Pro 340 345 350

Lys Gly Asp Ala Lys Pro Gly Ser Thr Gly Lys Ile Val Pro Leu His \$355\$

Ala Val Lys Val Val Asp Pro Thr Thr Gly Lys Ile Leu Gly Pro Asn 370 375 380

Glu Pro Gly Glu Leu Tyr Phe Lys Gly Pro Met Ile Met Lys Gly Tyr 385 390 395 400

Tyr Asn Asn Glu Glu Ala Thr Lys Ala Ile Ile Asp Asn Asp Gly Trp 405 410 415

Leu Arg Ser Gly Asp Ile Ala Tyr Tyr Asp Asn Asp Gly His Phe Tyr 420 425 430

Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln Val

Ala Pro Ala Glu Ile Glu Gly Ile Leu Leu Gln His Pro Tyr Ile Val 450 455 460 Asp Ala Gly Val Thr Gly Ile Pro Asp Glu Ala Ala Gly Glu Leu Pro 465 470 475 480

Ala Ala Gly Val Val Glr. Thr Gly Lys Tyr Leu Asn Glu Gln Ile 485 490 495

Val Gln Asp Tyr Val Ala Ser Gln Val Ser Thr Ala Lys Trp Leu Arg 500 505 510

Gly Gly Val Lys Phe Leu Asp Glu Ile Pro Lys Gly Ser Thr Gly Lys 515 520 525

Ile Asp Arg Lys Val Leu Arg Gln Met Leu Glu Lys His Thr Asn Gly 530 540

<210> 5

<211> 1639

<212> DNA

<213> Artificial Sequence

<220>

<223> Mutant of LucPpe2 luciferase

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720 ttotottgca aaagatoota ottttggtaa ogcaattaat oocacgacag caartttaac 780 ggtaatacct ttccaccatg gttttggtat gatgaccaca ttaggatact ttacttgtgg 840 attccgagtt gttctaatgc acacgtttga agaaaaacta tttctacaat cattacaaga ttataaagtg gaaagtactt tacttgtacc aacattaatg gcatttcttg caaaaagtgc 900 attagttgaa aagtacgatt tatcgcactt aaaagaaatt gcatctggtg gcgcaccttt 960 1020 atcaaaagaa attggggaga tggtgaaaaa acggtttaaa ttaaactttg tcaggcaagg 1080 gtatggatta acagaaacca cttcggctgt tttaattaca ccgaaaggtg acgccagacc gggatcaact ggtaaaatag taccatttca cgctgttaaa gttgtcgatc ctacaacagg 1140 aaaaattttg gggccaaatg aacctggaga attgtatttt aaaggcgcca tgataatgaa 1200 gggttattat aataatgaag aagctactaa agcaattatt gataatgacg gatggttgcg 1260 ctctggtgat attgcttatt atgacaatga tggccatttt tatattgtgg acaggctgaa 1320 gtcattaatt aaatataaag gttatcaggt tgcacctgct gaaattgagg gaatactctt 1380 1440 acaacatccg tatattgttg atgccggcgt tactggtata ccggatgaag ccgcgggcga gettecaget geaggtgttg tagtacagae tggaaaatat etaaaegaae aaategtaea 1500 agattttgtt tocagtcaag tttcaacagc caaatggcta cgtggtgggg tgaaattttt 1560 ggatgaaatt cccaaaggat caactggaaa aattgacaga aaagtgttaa gacaaatgtt 1620 1639 tgaaaaacac accaatggg

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<211> 1639

<212> DNA

<213> Artificial Sequence

<220>

<223> Mutant of LucPpe2 luciferase

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tgggacggct ggagaacaga tgtttgacgc attatctcgt tatgcagata ttccgggctg 120
catagcattg acaaatgctc atacaaaaga aaatgtttta tatgaagagt ttctgaaact 180

gtcgtgtcgt ttagcggaaa gttttaaaaa gtatggatta aaacaaaacg acacaatagc 240 ggtgtgtagc gaaaatggtc tgcaattttt ccttcctgta attgcatcat tgtatcttgg 300 aataattgtg gcacctgtta acgataaata cattgaacgt gaattaatac acagtcttgg 360 tattgtaaaa ccacgcatag ttttttgctc caagaatact tttcaaaaag tactgaatgt 420 480 aggttatcaa tgcctcaaca actttatttc tcaaaattcc gatagtaatc tggacgtaaa aaaatttaaa ccatattott ttaatogaga ogatoaggtt gogttgatta tgttttotto 600 tggtacaact ggtctgccga agggagtcat gctaactcac aagaatattg ttgcacgatt 660 ttctcttgca aaagatccta cttttggtaa cgcaattaat cccacgacag caattttaac 720 ggtaatacet ttecaccatg gtttrggtat gatgaccaca ttaggatact ttacttgtgg 780 attccgagtt gttctaatgc acacgtttga agaaaaacta tttctacaat cattacaaga 840 ttataaagtg gaaagtacti tacttgtacc aacattaatg gcatttcttg caaaaagtgc 900 attagttgaa aagtacgatt tatcgcactt aaaagaaatt gcatctggtg gcgcaccttt 960 atcaaaagaa attggggaga tggtgaaaaa acggtttaaa ttaaactttg tcaggcaagg 1020 gtatggatta acagaaacca cttcggctgt tttaattaca ccgaaaggtg acgccaaacc 1080 gggatcaact ggtaaaatag taccatttca cgctgttaaa gttgtcgatc ctacaacagg 1140 aaaaaattttg gggccaaatg aacctggaga attgtatttt aaaggcccqa tqataatqaa 1200 gggttattat aataatgaag aagctactaa agcaattatt gataatgacg gatggttgcg 1260 ctctggtgat attgcttatt atgacaatga tggccatttt tatattgtgg acaggctgaa 1320 gtcactgatt aaatataaag gttatcaggt tgcacctgct gaaattgagg gaatactctt 1380 acaacatccg tatattgttg atgccggcgt tactggtata ccggatgaag ccgcgggcga 1440 gettecaget geaggtgttg tagtacagae tggaaaatat etaaaegaae aaategtaca 1500 agattatgtt gccagtcaag tttcaacagc caaatggcta cgtggtgggg tgaaattttt 1560 ggatgaaatt cccaaaggat caactggaaa aattgacaga aaagtgttaa gacaaatgtt 1620 tgaaaaacac accaatggg 1639

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<220>

<223> Mutant of LucPpe2 luciferase

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<210> 8

<211> 1639

<212> DNA

<213> Artificial Sequence

<220>

<223> Mutant of LucPpe2 luciferase

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